Separation of 2,4-Dinitrophenyl-hydrazones of Aliphatic Aldehydes by Liquid-Liquid Extraction

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The easily prepared and stable 2,4-dinitrophenylhydrazones of carbonyl compounds are generally used for the isolation and characterization of the latter compounds. It is, however, very difficult to isolate hydrazones in pure form from their mixtures by crystallization because they readily form mixed crystals. Many good paper chromatographic methods have been developed, but they have only limited use in the separation of 2,4-dinitrophenyl-hydrazones. In this laboratory a cellulose powder column impregnated with dimethylformamide and the eluant *cyclo*hexane have been used for the separation of the 2,4-dinitrophenylhydrazones of the volatile carbonyl compounds of onion [1]. Good separations of the 2,4-dinitrophenylhydrazones of the lower aliphatic carbonyl compounds were effected by this technique, but it was not particularly effective for the higher homologs.

No counter-current method has been described for the separation of 2,4-dinitro-phenylhydrazones. The selection of a suitable solvent system in which the 2,4-dinitrophenylhydrazones would be soluble enough and their partition coefficients sufficiently different is difficult. The best solvent system found was petroleum ether (b.p. 80—100°C): ethylene glycol monomethyl ether ("methyl oxitol", a product from the Shell Co.): water (10:10:2.5). The partition coefficients of the 2,4-dinitrophenylhydrazones of the normal aliphatic aldehydes (C_1—C_9) in this solvent system are given in Table 1.

The distribution studies were carried out in a countercurrent apparatus described by Hietala [2,3]. This apparatus (available from E. Hanslin & Co., Kaisaniemenk. 10, Helsinki, Finland) consists of 400 tubes

Table 1. Partition coefficients at 20°C of the 2,4-dinitrophenylhydrazones of normal aliphatic aldehydes (C_1—C_9) in the solvent system petroleum ether (b.p. 80—100°): ethylene glycol monomethyl ether: water (10:10:2.5).

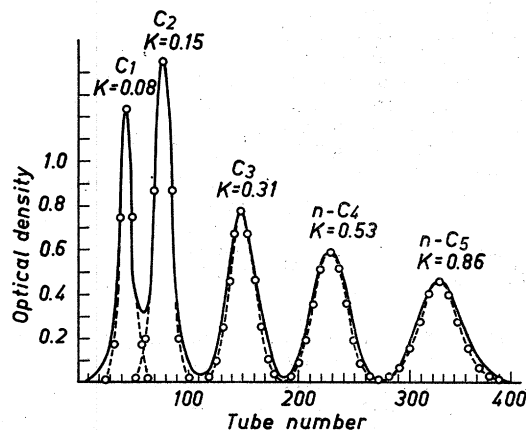| Aldehyde	K
C_1	0.08
C_2	0.15
C_3	0.30
$n\text{-}C_4$	0.52
$n\text{-}C_5$	0.83
$n\text{-}C_6$	1.3
$n\text{-}C_7$	1.9
$n\text{-}C_8$	3.1
$n\text{-}C_9$	4.5

Fig. 1. Distribution of 2,4-dinitrophenylhydzones of normal aliphatic aldehydes (C_1—C_5) in the solvent system: petroleum ether: ethylene glycol monomethyl ether: water, (10:10:2.5). ——— experimental curve, optical density of the bottom phase at 360 mμ. O— —O theoretical curve.
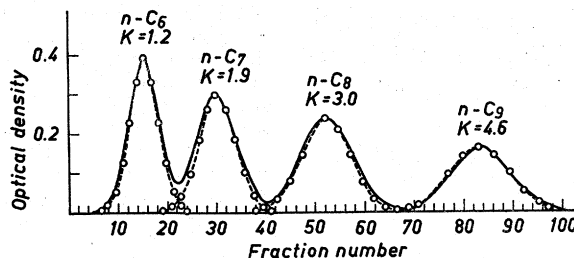
Fig. 2. Distribution of 2,4-dinitrophenylhydrazones of normal aliphatic aldehydes (C_6—C_9) in the solvent system: petroleum ether: ethylene glycol monomethyl ether: water (10:10:2.5)· ———— experimental curve, optical density at 360 mμ. O——O theoretical curve. Volume of each fraction 96 ml.

and operates with continuous flow of either the top or the bottom phase.

A mixture of 2,4-dinitrophenylhydrazones of the normal C_1—C_5 aldehydes (0.1 mmole of each) was distributed first. Each tube was filled with 4.3 ml of the top phase (v_t) and 9.0 ml of the bottom phase (v_b). The run was made at about 20°C with the top phase mobile and the solvent flowing at a rate of 1.0 ml/min. The shaking mechanism was adjusted to make 36 complete strokes per minute and the tilting angle was about ± 55°. After 4 850 ml (V_t) of effluent had emerged, a sample of the bottom phase was taken from every fifth tube and the light absorptions of the samples measured at 360 mμ with a spectrophotometer.

The resulting distribution curves are shown in Fig. 1. A complete separation of the five 2,4-dinitrophenylhydrazones was obtained. The theoretical distribution curves (the normal error curves) were calculated from the extinction values at the maxima. The partition coefficients calculated from eqn. (1) [3] corresponded very well with those obtained using a separatory funnel (Table 1).

$$K = \frac{v_b x_t}{V_t - v_t x_t} \quad (1)$$

V_t is the total volume (4 850 ml) of the effluent (top phase), v_t and v_b the volumes of the top and bottom phases, respectively, in one distribution tube, x_t the number of tubes through which the midpoint of the distribution of a substance has passed with the top phase.

For the separation of the higher homologs (C_6—C_9), a run was made with the bottom phase mobile. The phase volumes

were now v_t = 4.75 ml and v_b = 8.55 ml and the other factors the same as before. After 4 220 ml of effluent had emerged, fractions 96 ml in volume were collected until the 2,4-dinitrophenylhydrazone of n-nonanal had come out. The distribution curves are shown in Fig. 2. By this procedure a good separation of the 2,4-dinitrophenylhydrazones of the C_6—C_9 aldehydes was effected. The separation would have been even better if the volume of the top phase in each distribution unit had been larger (and the volume of the bottom phase correspondingly smaller). The partition coefficients in Fig. 2 were calculated from eqn. (2) [3].

$$K = \frac{V_b - x_b v_b}{x_b v_b} \quad (2)$$

V_b is the volume of the eluant needed to transport the midpoint of the distribution of a substance x_b (= 400) tubes.

Work on the separation of the 2,4-dinitrophenylhydrazones of the volatile carbonyl compounds of the flowers of red clover by the above liquid-liquid extraction technique is in progress.

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